

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claim 16 (currently amended): A process of clarifying a bacterial lysate comprising plasmid DNA and cellular debris, comprising the steps of:

- (a) introducing a gas into a fluid stream comprising a ~~suspended~~ bacterial cell suspension and a lysis buffer under conditions forming an entrainment of bubbles in the fluid stream during lysis;
- (b) admixing a precipitation buffer into the fluid stream, wherein the entrained bubbles generate a buoyant precipitate comprising the cellular debris;
- (c) allowing the buoyant precipitate to coalesce and separate over a fluid phase comprising the plasmid DNA; and
- (d) collecting the fluid phase comprising the plasmid DNA.

Claim 17 (original): The process of claim 16, wherein the lysis buffer is an alkaline lysis buffer.

Claim 18 (original): The process of claim 16, wherein the gas is introduced through an aperture comprising a plurality of pores of less than approximately 5 microns in diameter.

Claim 19 (original): The process of claim 18, wherein the pores are approximately 2 microns in diameter.

Claim 20 (original): A cell lysis process wherein cells in suspension are lysed in the presence of a controlled stream of gas bubbles sufficient to cause flotation and separation of a cellular debris component over a clarified lysate component comprising extrachromosomal nucleic acids.

Claim 21 (original): The process of claim 20 wherein the lysis process is an in-line process for alkaline lysis of bacterial cells and results in flotation of a cellular debris component following addition of a precipitation buffer.

Claim 22 (original): A method for the purification of extrachromosomal DNA from a pharmaceutical grade bacterial fermentation, comprising the steps of:

- (a) generating a fluidized stream of bacterial cells;
- (b) introducing a lysis buffer and a gas into the fluidized stream to form a cell lysate solution comprising a plurality of bubbles;
- (c) introducing a precipitation solution into the cell lysate solution wherein combined action of the bubbles and the precipitation solution results in the formation of a buoyant precipitate comprising cell debris and chromosomal DNA;
- (d) allowing the buoyant precipitate to coalesce and separate from an underlying fluid phase comprising the extrachromosomal DNA;
- (e) collecting underlying fluid phase to form a clarified lysate;
- (f) filtering the clarified lysate; and
- (g) subjecting the filtered clarified lysate to ion exchange chromatography to separate the extrachromosomal DNA from residual contaminants.

Claim 23 (original): A method of producing a clarified cell lysate comprising plasmid DNA from an alkaline bacterial cell lysate, comprising the steps of:

- introducing a suspension of bacterial cells into a fluid flow comprising an alkaline lysis buffer and an entrainment of gas, wherein the cells are flowably mixed with the cell lysis buffer together with the gas thereby forming a cell lysis mixture;
- introducing a precipitation buffer into the fluid flow comprising the cell lysis mixture, thereby forming a cell debris precipitate in the cell lysis mixture;
- separating the mixture into a buoyant flocculent phase comprising the precipitated cell debris and a fluid phase comprising a substantially clarified cell lysate; and
- isolating the substantially clarified cell lysate.

Claim 24 (new): The method of claim 23, wherein the gas is introduced via a gas port through which a gas is forced under pressure into the fluid flow thereby controllably forming bubbles in the cell lysis mixture.

Claim 25 (new): The method of claim 24, wherein the gas port comprises an aperture comprising a plurality of pores.

Claim 26 (new): The method of claim 25, wherein the pores have an average diameter of less than approximately 5 microns.

Claim 27 (new): The method of claim 25, wherein the aperture comprising a plurality of pores is a sparge stone or disk filter comprising pores having an approximate average diameter of 2 microns or less.

Claim 28 (new): The method of claim 23, wherein the fluid flow passes through one or more static mixers.

Claim 29 (new): The method of claim 23, further comprising introducing a pH adjustment buffer into the precipitated lysate and combining the pH adjustment buffer and the precipitated lysate prior to separating the mixture into the buoyant flocculent phase and the fluid phase comprising a substantially clarified cell lysate.

Claim 30 (new): The method of claim 23, wherein the separation of the mixture into a buoyant flocculent phase and a fluid phase is performed in a lysate separation tank.

Claim 31 (new): The method of claim 23, wherein the fluid flow is a contained continuous flow fluid path.

Claim 32 (new): A method of producing a clarified cell lysate comprising plasmid DNA from an alkaline bacterial cell lysate, comprising the steps of:

introducing a suspension of bacterial cells into a fluid flow comprising an alkaline lysis buffer and an entrainment of gas and flowably mixing the cells with the cell lysis buffer together with the gas by passage through a first static mixer, thereby forming a cell lysis mixture;
introducing a precipitation buffer into the fluid flow comprising the cell lysis mixture and flowably mixing the cell lysis mixture with the precipitation buffer by passage through a second static mixer, thereby forming a cell debris precipitate in the cell lysis mixture;
introducing a pH adjustment buffer into the fluid flow comprising the cell debris precipitate and flowably mixing the cell lysis mixture with the pH adjustment buffer by passage through a third static mixer, thereby forming a pH adjusted cell lysis mixture;
flowing the pH adjusted cell lysis mixture into a lysate separation tank for separating the cell lysis mixture into a buoyant flocculent phase comprising the precipitated cell debris and a fluid phase comprising a substantially clarified cell lysate;
obtaining the substantially clarified cell lysate from under the buoyant flocculent phase; and
filtering the substantially clarified cell lysate to form a clarified cell lysate.

Claim 33 (new): The method of claim 32, wherein the gas is introduced via a gas port through which a gas is forced under pressure into the fluid flow thereby controllably forming bubbles in the cell lysis mixture.

Claim 34 (new): The method of claim 33, wherein the gas port comprises an aperture comprising a plurality of pores.

Claim 35 (new): The method of claim 34, wherein the pores have an average diameter of less than approximately 5 microns.

Claim 36 (new): The method of claim 34, wherein the aperture comprising a plurality of pores is a sparge stone or disk filter comprising pores having an approximate average diameter of 2 microns or less.